REMARKS/ARGUMENTS

The Pending Claims

Claims 1-12, 15, 16, 35, and 36 are pending and are directed to a method of producing pluripotent stem cells.

Amendments to the Claims

The claims have been amended to point out more particularly and claim more distinctly the invention. In particular, claims 1, 8, and 12 have been amended to recite that the culturing period is at least 3 to 6 weeks, as supported by the specification at, for example, page 25, lines 22-32, and page 26, lines 22-27.

Claims 35 and 36 are new and recite that the testis cells are derived from a rodent, such has a mouse. Support for claims 35 and 36 can be found at, for example, page 15, lines 12-20, of the specification.

Accordingly, no new matter has been added by way of these amendments to the claims.

Summary of the Office Action

The Office rejects claims 1-12, 15, and 16 under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement.

The Office rejects claims 1, 2, 4-6, and 15 as allegedly obvious under 35 U.S.C. § 103(a) over Hogan (U.S. Patent 5,690,926) and Creemers et al. (*Reproduction, 124:* 791-799 (2002)). In addition, the Office rejects claims 3, 7, and 16 as allegedly obvious under 35 U.S.C. § 103(a) over Hogan and Creemers et al. and further in view of one or more of the following: (i) Haneji et al. (*J. Endocrinol., 128(3)*: 383-388 (1991)), (ii) Wahab-Wahlgren et al. (*Mol. Cell. Endocrinol., 201*: 39-46 (2003)), (iii) Beumer et al. (*Cell Death and Differentiation, 5*: 669-677 (1998)), (iv) Kanatsu-Shinohara et al. (*Biol. Reprod., 69*: 612-616 (2003)), (v) Shinohara et al. (*Biol. Reprod., 66*: 1491-1497 (2002)), and (vi) Van Der Wee et al. (*J. Androl., 22(4)*: 696-704 (2001)).

Reconsideration of the rejections is hereby requested.

Information Disclosure Statement

Applicants respectfully request that the Office confirm consideration of References AM-AR submitted with the Information Disclosure Statement dated July 29, 2009, and Reference AS submitted with the Information Disclosure Statement concurrently filed herewith, by providing Applicants with appropriately marked copies of the Forms PTO-1449.

Discussion of the Enablement Rejection

The Office alleges that the pluripotent stem cells produced from testis cells derived from a postnatal mammal including younger mammals with higher frequencies of stem cells (e.g., spermatogonial stem cells) embrace pluripotent stem cells derived from embryonic stem cells (see page 3, lines 5-8, of the Office Action). Applicants respectfully disagree with the Office's contention, which Applicants believe is based on an insufficient understanding of the claimed invention.

The claimed method comprises the derivation of pluripotent stem cells from postnatal testis cells comprising spermatogonial stem cells under specific conditions. Embryonic stem cells (ES cells) are entirely different from the spermatogonial stem cells recited in the pending claims. ES cells are well-known pluripotent stem cells produced by *in vitro* cultivation of an inner cell mass. The guidance provided in the specification regarding the age of the postnatal mammal makes it clear that the postnatal mammal does not contain ES cells (see, e.g., page 15, line 34, through page 16, line 22, of the specification).

The specification describes the problems associated with the production of pluripotent stem cells from adult testis and specific methods to solve the problems. In particular, the specification identifies the low frequencies of stem cells in adult testis as a significant problem to be solved in the production of pluripotent stem cells from adult testis cells. The specification provides specific guidance as to methods of solving this problem by using a younger animal because younger animals have higher frequencies of stem cells (e.g., spermatogonial stem cells) contained in the testis (see, e.g., page 14, line 23, through page 15, line 5, and page 16, line 3-6), and using a cell sorter or antibody magnetic microbeads with an antibody-recognizing cell surface antigen specifically expressed in the spermatogonial stem cells (see, e.g., page 14, line 29, through page 15, line 2).

Applicants previously cited to Guan et al., *Nature, 440:* 1199-1203 (2006), Guan et al., *Nature Protocol, 4*: 143-154 (2009), and Conrad et al., *Nature, 456*: 344-351 (2008), as evidence of the success of the inventive method. The Office contends that the Guan and Conrad references do not teach the derivation of pluripotent stem cells containing ES cells. As discussed above, the claimed method comprises the derivation of pluripotent stem cells from postnatal testis cells comprising spermatogonial stem cells under specific conditions. ES cells are entirely different from the spermatogonial stem cells recited in the pending claims. Therefore, the pending claims do not encompass the derivation of pluripotent stem cells from ES cells.

The Guan 2006 reference discloses that testis cells containing Stra8 positive spermatogonial stem cells from adult mice cultured in a medium containing GDNF result in the formation of testicular teratomas (tumors derived from pluripotent cells) at low frequencies (see, e.g., page 1199, column 1, lines 23-41).

The Guan 2009 reference discloses the isolation of testis cells from 2 to 5 week old mice (see page 143, "Isolation of testicular cells"), the enrichment of spermatogonial stem cells from testis cells in various ways (see page 143, "Methods for enriching for SSCs"), culturing in a medium containing GDNF (page 143, "Culturing SSCs"), and the appearance of ES-like colonies of maGSCs (pluripotent stem cells) at 113 days (or at least 4-8 weeks) after initiation of the culture (see page 149, "Conversion of SSCs into maGSCs" and Figure 2C).

The Conrad reference discloses the enrichment of spermatogonial cells from adult human testis using MACS with an antibody against α6-integrin, culturing in a medium containing GDNF and LIF or GDNF alone for as long as 42 days to develop clusters of GSCs (pluripotent stem cells), and the formation of teratomas after injection of the resultant human adult GSCs into an immunodeficient mouse (see, e.g., Figures 2 and 5, and page 5, column 1, lines 13-30).

Accordingly, the post-filing Guan and Conrad references further evidence the success of the inventive method using adult mice and human testis cells.

Applicants previously cited to Kossack et al., *Stem Cells, 27*: 138-149 (2009), as evidence of the success of the inventive method; however, upon further consideration, Applicants note that the culture medium used in the method described in the Kossack reference does not contain GDNF (see page 139, "Transfer and Culture Conditions of Spermatogonial Stem Cell Colonies"). Therefore, the Kossack reference does not have all of the features of the claimed invention. For this reason, Applicants believe that the Office's concerns regarding the Kossack reference are not relevant to the claimed invention.

Applicants previously pointed out that one of ordinary skill in the art at the time of filing the application would have been able to easily extrapolate the production conditions of human pluripotent stem cells from the production conditions of mouse pluripotent stem cells given the teachings in the prior art, such that no undue experimentation would be required to practice the inventive methods. Applicants cited to Hogan (U.S. Patent 5,690,926), which demonstrates that pluripotent stem cells can be produced from human primordial germ cells under basically the same conditions as mouse embryonic germ cells (e.g., stem cell factor (SCF), basic fibroblast growth factor (bFGF), and leukemia inhibitory factor (LIF)) (see column 12, lines 14-60). The Office contends that Applicants' arguments were in part convincing for the derivation of pluripotent stem cells from spermatogonial stem cells, but not for the derivation of pluripotent stem cells from ES cells derived from the inner cell mass. As discussed in detail above, the inventive method does not encompass the derivation of pluripotent stem cells from ES cells.

Regarding Turnpenney et al., *Stem Cells, 24*: 212-220 (2006), the Office alleges that the culture of embryonic germ (EG) cells from different species is controversial and unpredictable. The Office believes that the proliferation of mouse spermatogonial stem cells is dependent on LIF and not GDNF. Applicants reiterate that the culture conditions for human pluripotent stem cells do not differ much from the culture conditions for mouse pluripotent stem cells. The Guan 2009 reference teaches derivation of mouse pluripotent stem cells from spermatogonial stem cells by culturing mouse spermatogonial stem cells in a medium containing GDNF alone (i.e., without LIF) (see page 147, "Culture of SSCs," and page 149, "Conversion of SSCs into maGSCs"). The Conrad reference teaches derivation of human pluripotent stem cells from spermatogonial stem cells by culturing human spermatogonial stem cells in a medium containing GDNF alone (i.e., without LIF) (see pages

1-3, "Generation of pluripotent human adult GSCs," and Figure 2). Thus, there is no difference in the external factors required (i.e., GDNF and not LIF) for the derivation of pluripotent stem cells from spermatogonial stem cells between mouse and human. Additionally, the Hogan reference demonstrates that pluripotent stem cells can be produced from human primordial germ cells under basically the same conditions as from mouse embryonic germ cells.

The Office previously alleged that Aflatoonian et al., *Curr. Opin. Biotech., 16*: 530-535 (2005), describes the difficulty of maintaining well-defined human EG cell lines through extended passage in culture, even though the initial generation of human EG cells is relatively simple. Applicants note that the pending claims are directed to a method of producing (i.e., generating) pluripotent stem cells from testis cells, and not to a method of maintaining human EG cells in culture over multiple passages. However, even if the maintenance of human EG cells is difficult, Applicants note that human EG cells have been established as described in Turnpenney et al., *Stem Cells, 21*: 598-609 (2003).

For the above-described reasons, the specification provides adequate enablement for the inventive methods, such that one of ordinary skill in the art at the time the application was filed would understand how to use the inventive methods with a reasonable expectation of success and without undue experimentation. Therefore, Applicants request that the enablement rejection be withdrawn.

Discussion of the Obviousness Rejections

The Office contends that it would have been obvious to one of ordinary skill in the art to arrive at the inventive methods based on the disclosures of the cited references. The obviousness rejections are traversed for the following reasons.

For subject matter defined by a claim to be considered obvious, the Office must demonstrate that the differences between the claimed subject matter and the prior art "are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains." 35 U.S.C. § 103(a); see also *Graham v. John Deere Co.*, 383 U.S. 1, 148 U.S.P.Q. 459 (1966). The ultimate determination of whether an invention is or is not obvious is based on certain factual inquiries including: (1) the scope and content of the prior art, (2) the level of

ordinary skill in the prior art, (3) the differences between the claimed invention and the prior art, and (4) objective evidence of nonobviousness. *Graham*, 383 U.S. at 17-18, 148 U.S.P.Q. at 467.

Consideration of the aforementioned *Graham* factors here indicates that the present invention, as defined by the pending claims, is unobvious in view of the cited references.

As regards the scope and content of the prior art, the Office contends that the Hogan reference discloses a method of making mammalian pluripotent ES cells by culturing postnatal mammalian testis in a composition comprising bFGF, LIF, membrane associated steel factor, and soluble steel factor, thereby making a pluripotent ES cell from a germ cell. The Office also contends that the Hogan reference teaches isolating the ES cells from postnatal mammalian testis. The Office acknowledges that the Hogan reference does not teach the use of GDNF; however, the Office contends that the Creemers reference teaches culturing spermatogonial cells in a medium containing GDNF, LIF, and bFGF. The Office contends that it would have been obvious for one of ordinary skill in the art to add GDNF to the culture system of the Hogan reference because the Creemers reference suggests that optimization of the culture medium could improve viability or proliferation of spermatogonia. The Office relies on the remaining cited references to provide the features of the remaining dependent claims.

For purposes of the analysis here, and for the sake of argument, the level of ordinary skill can be considered to be relatively high, such that a person of ordinary skill in the art would have an advanced degree and/or several years of experience in the relevant field.

The present invention, as defined by the pending claims, is directed to a method of producing pluripotent stem cells, which comprises culturing testis cells using a medium containing GDNF or an equivalent thereto for at least 3 to 6 weeks, wherein the testis cells contain spermatogonial stem cells, and wherein the testis cells are derived from a postnatal mammal, and isolating pluripotent stem cells from the cultured testis cells.

The Creemers reference discloses culturing type A spermatogonia in KSOM medium containing LIF, bFGF, and GDNF. However, the culture period in the method disclosed in the Creemers reference is 7 days at the longest (see Figures 2 and 5). The Creemers reference

does not teach or suggest culturing spermatogonia for at least 3 to 6 weeks, as required by the pending claims. Indeed, the Creemers reference teaches away from longer culture periods. In particular, the Creemers reference discloses that viability of type A spermatogonia decreases to about 20% after culture for 7 days in KSOM medium containing LIF and bFGF (see Figures 2, 3, and 5). The Creemers reference indicates that the addition of GDNF does not significantly influence the viability of the type A spermatogonia (see page 794, column 1, lines 37-40). Based on the teachings of the Creemers reference, one of ordinary skill in the art would not have had a reason to culture the spermatogonial stem cells for more than 7 days using the culture conditions of the Creemers reference to grow the spermatogonial stem cells, let alone for the "at least 3 to 6 weeks" required by the inventive method as defined by the pending claims.

The Creemers reference cultures a mixture of type A spermatogonia of various stages (As to A) (see page 792, column 2, 5th line from the bottom), focuses on the viability of the total mixture of the type A spermatogonia, and demonstrates that the viability of the mixture decreases after culturing for 7 days. Applicants note that it would not be possible for one of ordinary skill in the art to elucidate the effect of the culture condition on the growth of spermatogonial stem cells by analyzing the viability of the total mixture of type A spermatogonia since the number of spermatogonial stem cells in the type A spermatogonia is very small and the majority of the type A spermatogonia is non-stem cells.

None of the remaining cited references provides any credible reason for one of ordinary skill in the art to increase the culturing time in the method of the Creemers reference.

Therefore, one of ordinary skill in the art given the teachings of the Creemers reference would not have tried to apply GDNF to the culture system of the Hogan reference and culture the postnatal testis cells in the presence of GDNF for at least 3 to 6 weeks in order to produce pluripotent stem cells from testis cells, as required by the pending claims, because one of ordinary skill in the art would not have had a reasonable expectation of success (in view of the teachings in the Creemers reference about decreased viability with longer culturing times).

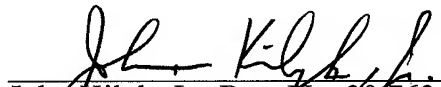
The inventors surprisingly discovered that when testis cells containing spermatogonial stem cells are cultured in a medium containing GDNF for at least 3 to 6 weeks, spermatogonia other than spermatogonial stem cells may die, but spermatogonial stem cells can grow to form colonies of two different morphologies including colonies of GS cells and colonies of pluripotent stem cells. None of the cited references even hints at such a surprising result.

Considering all of the *Graham* factors together, it is clear that the present invention – as defined by the pending claims – would not have been obvious to one of ordinary skill in the art at the relevant time in view of the combined disclosures of the cited references. Accordingly, the obviousness rejection should be withdrawn.

Conclusion

Applicants respectfully submit that the patent application is in condition for allowance. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned attorney.

Respectfully submitted,



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